Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.25, 2014
www.iiste.org

Impact of Processing Techniques on Nutrient and Anti-Nutrient Content of Grain Amaranth (*A. albus*)

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Abstract
Grain amaranth (*Amaranthus albus*) is a pseudo cereal consumed in various parts of the world especially in Africa, India, Nepal and some non-native regions. It has attracted increasing interest over recent decades because of its nutritional and functional properties. Reducing anti-nutrients level in the grain prior to consumption can increase the bioavailability of nutrients. Therefore, this calls for increased efforts towards evaluation of how different processing technologies can improve the bioavailability and absorption of nutrients. Traditionally, amaranth grain is boiled, popped, roasted, or milled to make gruel for consumption. This study investigated the impact of dry and wet heat processing techniques on the nutrient and anti-nutrient contents of grain amaranth. Proximate composition and anti-nutrients content were determined using standard procedures. Protein digestibility was determined after enzyme digestion while starch gelatinization was evaluated using light microscopic techniques The dry heating processes used included roasting(160ºC 10min) and popping(190ºC 15s)while the wet heating techniques included boiling whole grains and slurries(100ºC 25min). Generally, the effects of dry and moist heat processing with regards to loss and retention of the nutrients differed significantly (p>0.05), with only the roasting retaining more of the nutrients than boiled seed flour. A pronounced reduction in the anti-nutrient content (tannins, oxalates, and phytates) was observed in the case of boiling as compared to roasting and popping. Boiling and slurring resulted in, 86.2% and 92.6% increase in protein digestibility respectively whereas roasting reduced it to 66.3% as compared to raw grains (74.8%). Dry heating resulted in partial gelatinization of starch unlike wet processing which showed complete gelatinization. These results indicate that wet processing techniques are superior to dry processing techniques in terms of nutrient bioavailability
Keywords: Grain amaranth, gelatinization, protein digestibility, anti-nutrient

1.0 Introduction

Amaranth is one of the few multi-purpose crop which can supply grains and tasty leafy vegetables of high nutritional quality as a food and animal feed, and additionally, because of attractive inflorescence coloration, amaranth can be cultivated as an ornamental plant. Although the crop was one of the staple foods in the pre-colonized South American civilizations, the cultivation and production of grain amaranth were given less consideration and thus nowadays it can be classified as a forgotten, neglected and alternative crop of great nutritional value (Earnest, 2009). The poor nutritional values of the few, but most produced, crop species in the world today and consecutively occurred erosion of cultivated land are some of the reasons for renewed interest in alternative crops. The use of alternative crops would result in product competitiveness and diet diversity and thus boost productivity (Bavec and Bavec 2006).

Amaranth (family Amaranthaceae) is an underexploited plant with an exceptional nutritive value. Amaranths are broad-leafed non-grass plants that are easy to grow, nutrient rich and an underutilized pseudo cereal that can play an important role in actions against hunger and malnutrition. It is either consumed as a leafy vegetable or as grain. The grain amaranth is very versatile as a food ingredient and can diversify farming enterprise; as it can be used to prevent food depletion and to feed the world (Saunders and Becker 1984).

Amaranth has been touted as a miracle grain, a super grain, and the grain of the future (Samuel, 1991; Evgeny, 2001). In Kenya, improved seeds have led to increased production of grain amaranth in Western and Nyanza region.

The seeds contain large amounts of dietary fiber, iron, and calcium. The crude protein content of grain amaranth ranges from 11 to 17.6 % dry matter (Bressani *et al.*, 1987 a; Imeri *et al.*, 1987 b; Bressani *et al.*, 1987 b). This is higher than in most common grains except soybeans. The protein quality of grain amaranth is complete containing around 5% lysine and 4% sulphur amino acids, which are the limiting amino acids in other grains. The lysine content is given as the main reason for the high protein quality of amaranth (Saunders *et al.*, 1983; Teutonico and Knorr, 1985). Amaranth complete protein also contains significantly more sulphur amino acids than soya complete protein. The amino acid composition of amaranth protein compares well with the FAD/WHO protein standard for instance lysine in amaranth is 6.233g/100g while the standard 5.4g/100g. In addition to its outstanding nutritional value, amaranth is also very low in sodium and contains low saturated fat (Garuda, 2004, Kariuki *et al* 2013)

Grains can be roasted, popped, while whole grains can be boiled and mixed with various foods like rice, steamed vegetables and other dishes to boost nutrient level or be ground to produce flour which can be used to prepare gruel for consumption (Muyonga *et al*., 2008)

In Kenya, government and non-government organizations are currently working on the design and implementation of actions to mitigate hunger and malnutrition that affects vulnerable groups. Amaranth has been picked by the ministry of planning, poverty eradication commission as one of the crops that could serve as a vehicle for meeting the above objective. In this context, this study evaluated the impact of different thermal processing techniques on the nutrients and anti-nutrient value of grain amaranth. The results could form a basis for tailoring the chemical properties of raw material to meet target consumer specifications.

2. Materials and Methods

2.1 Research design

Six kilogram of grain amaranth (*Amaranthus albus*) seeds which were harvested at different seasons were collected from Jomo Kenyatta University of Agriculture & Technology farm. They were subjected to cleaning prior to storage for subsequent processing techniques and analyses. Complete randomized block design was used in carrying out the data collection. Processing techniques were applied w as blocks were the blocks and anti-nutrients and nutrients composition were the treatments.

2.2 Sample preparation and processing

Clean grains were subjected to different processing techniques. They were subjected to dry heat techniques (roasting 160^{0}C 10minutes and popping 190^{0}C 5s) and moist heat techniques (boiling whole grains 100^0 C 30min ration of seeds to water 1:4 and slurring 100^{0}C 25 min) ration of flour to water 1:6 The effect of these processing techniques on nutrients and anti-nutrients content was determined. For each treatment, three samples were analyzed each in triplicate. Analysis was carried for raw and processed samples for comparison purposes.

2.3 Determination of changes in nutritional characteristics

Proximate composition and total carbohydrate were determined using standard procedures were determined using (AOAC methods (1995) The Zn, Fe, Cu, Ca and Mg content were determined by Atomic Absorption Sphectrophotometry, while Na and K were determined by Flame Atomic Spectrophotometry. Total carbohydrate content of the samples was determined by subtracting method (Pearson, 1976).

2.4 Determinations of changes in anti-nutrient factors

Analysis of phytic acid in grain amaranth was done by HPLC combining the column/mobile phase conditions established by Tanjendjaja *et al.,* (1980), with modification as detailed by Camire and Clydesdale (1982***).*** Tannins were determined by the Folins- Denis colorimetric method described by Kirk and Sawyer (1998). The analysis of oxalates was determined according to Libert (1981) with modifications (Yu et al., 2002).

2.5 Protein digestibility and starch gelatinization

Protein digestibility was determined according to the method by Hamaker et al (1987) whereby the sample was digested in 2ml of pepsin for 2hours at 37ºC. Degree of starch gelatinization was determined by Light microscopy with iodine staining as stipulated by Ophardt (2003)

2.6 Data analysis

Each determination was carried out on triplicates, the figures were then averaged and standard deviation calculated. Data was assessed using ANOVA with Genstat. Mean comparisons were made using Duncan's Multiple Range Tests (Steel and Torrie, 1980).

3. Results and discussion

3.1 *Changes in nutritional characteristics of grain amaranth during processing*

The gross chemical composition of raw and processed grain amaranth is shown in **Table 1** which has been reported in wet weight basis. The data showed that raw grain amaranth is high in protein, ash, fat and less in crude fibre. The results of raw grains are closely related to what has been reported by other researchers.

Moisture content of raw grains was slightly lower than the reported values (9.74 vs. 11-13%) (Table1). This may have been attributed by extension of drying period of the grains prior to storage. This Chemical compositions help in inhibiting the enzymes activity and growth of moulds. Chemical composition of grain amaranth subjected to different processing methods has been shown in Table 1. Dry heat techniques showed reduction of moisture content, but roasting had less moisture content as compared to popping. This could be as a result of the high temperature and time the grains were subjected to and significantly differed to moisture or raw grains. Moist heat techniques increased the moisture content of the seeds and flour respectively due to absorption of water during cooking (Mubarak, 2005)

Table 1: Proximate composition of raw and processed grain amaranth (mg/100g)

Parameters	Moisture content	Crude protein	Crude fat	Crude ash	Crude fiber	CHO
Raw grain	**9.74**	**14.44**	**7.09**	**3.18**	**4.27**	**66.28**
Popped grain	**6.38**	**14.35**	**6.87**	**3.07**	**3.36**	**65.17**
Roasted grain	**5.78**	**14.15**	**7.00**	**2.88**	**4.09**	**64.95**
Boiled grain	**73.99**	**3.53**	**1.69**	**1.32**	**2.09**	**17.38**
Slurry	**86.37**	**2.81**	**1.34**	**0.88**	**1.57**	**7.03**

Means are represented in the column

The crude protein was decreased after processing as compared with raw grains. These results are agreed with Shaker *et al.* (1995) who reported that nutrients loss might be attributed to the leaching of soluble nitrogen, mineral and other nutrients into desired solution especially in boiling. Similar reports had been given earlier for boiled soybean and mungbean flour as well as fluted pumpkin seed flour (Kylen and McCready, 1975; Fagbemi, 2007). Roasting reduced crude protein content from 14.4 to 14.15%. This result may be due to high temperature of roasting, being a dry heat processing method as well as prolonged tome of roasting. Similar report was made by Fagbemi (2007) on toasted fluted pumpkin seed flour.

Crude fat of the raw sample (7.09%) fell within the range (5.6 to 10.9%) reported by Mlakar *et al* 2009 Kariuki *et al.*, 2013 and it differed significantly from processed samples. Moist heat processing methods decreased fat content. Mubarak (2007) reported reduction of fat content on mungbean seeds and the decrease was attributed to their diffusion into cooking water. Furthermore, Ukwuru (2003) observed a reduction in the fat content of cooked soy flour to be due to leaching into boiling water.

Crude ash of raw grain amaranth (3.18%) is within the range (2.5 to 4.4%) reported by Mlakar *et al.,* (2009). However moist heat processing has shown reduction of ash content, particularly boiling due to leaching of soluble minerals into the cooking water. Low levels of ash content in slurried samples are attributed to dilution factor of cooking water.

The decrease in crude fibre content of the moist heated sample was attributed to loss of solid particles by boiling dry heat techniques (Albercht *et al*., 1966).

The result of mineral contents of raw and processed grain amaranth (*A. albus)* has been shown in **Table 2.** Results indicated that grain amaranth is a rich source of both major and trace elements. In general, cereals high in phytate content tend to have higher iron content. Moist heat has got lower minerals as compared to dry heat techniques. The reason why there is low content of minerals in slurried samples is due to dilution effect during its preparation.

Table 2: The mineral composition of raw and processed sample of grain amaranth (mg/100g)

parameters	Raw grain	Popped grains	Roasted grains	Boiled grains	slurry
Fe	**35.02**	**23.90**	**18.12**	**15.50**	**7.26**
Mg	**653.27**	**578.49**	**546.44**	**473.73**	**326.78**
Ca	**578.24**	**562.53**	**545.15**	**374.75**	**318.86**
Zn	**5.33**	**5.151**	**3.61**	**1.79**	**1.35**
Cu	**0.916**	**0.83**	**0.81**	**0.46**	**0.40**
K	**729.69**	**714.98**	**720.54**	**620.54**	**604.86**
Na	**94.537**	**90.861**	**87.96**	**69.67**	**65.42**

Values in rows marked with different letters are significantly different at $\alpha = 0.05$.

Comparatively, it has been observed that moist heat techniques particularly boiling decreased the mineral content more than the dry heat method. This occurred probably because soluble minerals leached into the processing water which was decanted. This result agreed with the research of Fox and Cameron (1984) and Edem *et al.* (1994) that soluble minerals get lost by dissolving into cooking water which is normally drained off. The low content of minerals in slurry could be attributed by dilution of dry matter.

3.3 Anti-nutritional factors

Raw grains had the phytates content amounting to around 0.796mg/100g. The phytates content of the raw grain is in the range that has been reported. Whittaker and Ologunde (1990) Ruiz and Bressani (1990) and Matz (1991) reported that phytate content in raw amaranth cereal ranged 0.29- 7.92 mg/g. Generally, all the processing techniques reduced the phytates contents in grain amaranth. Roasting and popping had slight reduction of phytates content, while boiling and slurring ha great reduction of phytates. From Figure 1 it has been clearly shown that moist heat processing had greater impact in reduction of phytates concentrations as compared to dry heat techniques.

The effect of different thermal processing methods on the levels of anti-nutritional factors has been shown in Figure 1. All the thermal processing methods reduced tannins, phytates and oxalates. However, the reduction of anti-nutrients was highest with moist heat (91 to 57.7 mg/100g for oxalates, 0.304 to 0.206mg/100g for tannins). The reduction concentrations for popped and roasted grain amaranth were almost similar for both processes.
Roasting was found to be least effective method for reducing anti-nutrients content. The roasting of grain amaranth decreased the oxalates content by 56.1%. Around 80.9% of total oxalates were lost when grain amaranth were slurried compared with boiled (69%) and popped (66.4%)

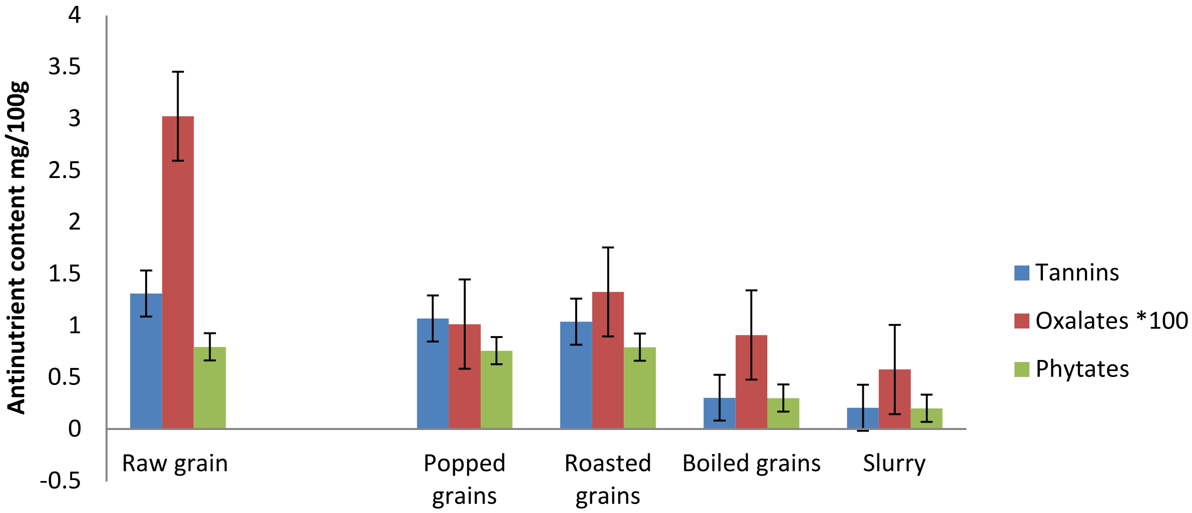

Figure 1: Anti-nutrient contents of grain amaranth (mg)

Thermal processing of grain amaranth is acknowledged to be very successful in enhancing the nutritional value of grain amaranth and in reducing these anti-nutrient factors. These processes are however affected by many and varied factors especially the influence of temperature-time combinations on the reduction anti-nutrient factors.
Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet cooking may reduce oxalates more rapidly when compared with just wet cooking (Hotz & Gibson, 2001).

3.3 Protein digestibility

The protein digestibility of raw and processed grain amaranth varied with the processing method (Figure 2). Raw grain had a protein digestibility of 74.8% Dry heat (popping) and moist heat (boiling and slurring) improved protein digestibility (78.04%, 86.19% and 92.55%) respectively unlike roasting which was marked by a pronounced reduction in protein digestibility.

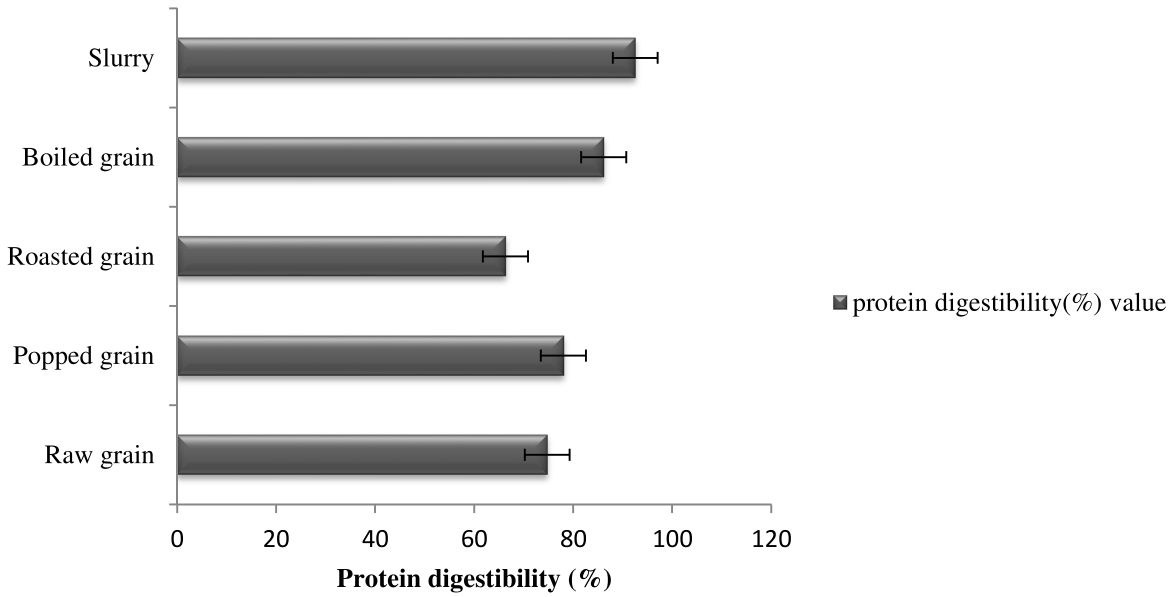

Figure 2: protein digestibility (%) of raw and processed grain amaranth

It has been reported that the protein digestibility of the raw complete amaranth protein is in the range 74 - 80 % which is in the range of obtained value. However, the digestibility and the protein efficiency ratio are significantly improved if the grain is heat processed (Garcia *et al*., 1987). During heat treatment, trypsin inhibitors and other anti-nutritional substances are denatured (Imeri *et al*., 1987).

The relatively low protein digestibility of raw grain amaranth may be attributed to the influence of anti-nutrients such as enzyme inhibitors, lectins, phytates, tannins and dietary fiber, which inhibits protein digestion, and also due to presence of protein structures that resist digestion.

The digestibility of popped is higher than in raw grain, but lower than in wet cooked grain and gruel. This is probably due to unfolding of the proteins during protein denaturation, thus exposed more proteins for enzyme digestion, also there was reduction of anti-nutrient factors that mostly inhibit digestion of proteins by enzymes. The low values obtained for roasted amaranth could have been attributed by prolonged period of heating of grains during roasting; that lead to maillard reaction and hence rendered most proteins unavailable for digestion.

It has been shown that wet cooking improved the protein quality the most as compared to dry cooking. The slurry had the highest protein digestibility as compared to all processing techniques; this is because there is size reduction of grains attributed by milling to flour thus increasing surface area exposed for enzyme digestion. Popping and moist heat processing may have contributed to denaturation of proteins. This exposed most of the proteins for enzyme digestion in relation to roasting.

3.5 Starch gelatinization

By light microscopy, the granules of raw grain amaranth showed polygonal shape (plateI), whereas dry heat showed some loss of cavities (plate II and plate III) and moist heat showed great irregularity of cavities (plate IV and plate V) which resulted from imbibitions of water.

Changes in starch during processing have been extensively studied for human food applications (Hellendoorn *et al*., 1975).Most starches will gelatinize upon heating to above 80^0C in excess water. Gelatinization markedly increases susceptibility for amylolytic degradation due to loss of crystalline structure. Gelatinization has been described as a swelling driven process.

Swelling occurs along the amorphous regions, and since the crystalline regions do not expand during swelling, stress increases at the interface between the crystalline and amorphous regions, where bonds exist between amylopectin in the crystalline regions and amylose in the amorphous regions. Thus, at a certain point in the swelling process, the crystalline regions are rapidly and irreversibly broken and gelatinization is initiated (Fennema, 1996)

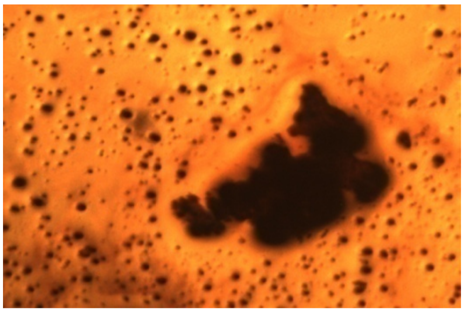

Plate I: Raw grain

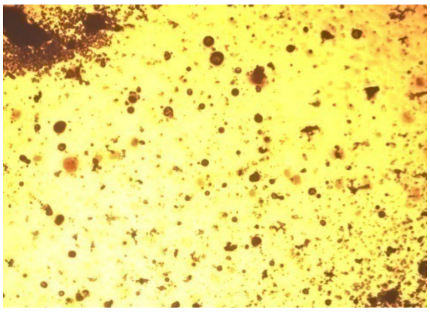

Plate II: Roasted grains

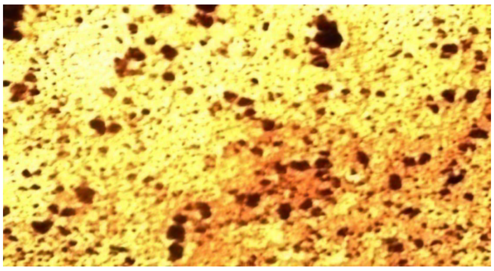

Plate III: Popped grains

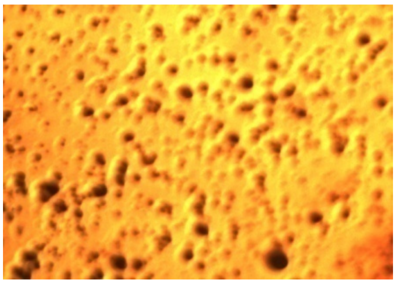

Plate IV: Boiled grains

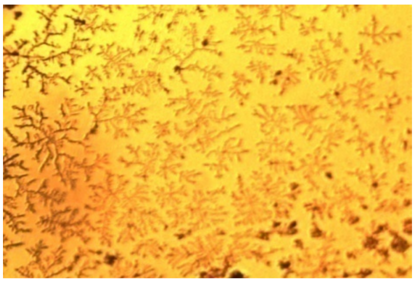

Plate V: Slurried sample

Moist heat (boiling and slurring) causes the starch grains to swell so that the coating of cellulose is broken and softened. After being softened and swollen, the starch granules become gelatinized. This may take place below -the boiling point but most starches in various cereals are more thoroughly cooked by high temperatures. Raw starches cause much digestive trouble. At excess water content, this onset of the gelatinization usually occurs between 50 and 70^{0}C. Swelling causes nearly all amylose in the starch granule to leach out.

Dry heat (popping and roasting) changes starch to dextrin, a form of sugar that is very soluble. Popping is achieved by rapid, intense heating of grain; it makes water expand all at once; thereby expanding the grain. As expansion takes place, some of the granules are gelatinized resulting in the grain being much more available to digestive enzymes. In roasting, water is lost without the expansion of the grain; this is because grains are heated at a much slower rate than the popping. This results to partial gelatinization of starch. This change takes place in the crust of bread, and for this reason, the crust is more digestible than the center portion of a loaf.

3.6: Fatty acid profile

The oil extracted from amaranth grain contains mainly unsaturated fatty acids (**Figure 3**). The predominant acids in the oil are oleic, linoleic and palmitic. Total unsaturated acids of raw grain fall within the range of reported values 74.4 % and saturated fatty acids 25.6 % Linolenic acid was present at low concentration. The percent unsaturation in amaranth fats is reported as 65 -76% by Saunder and Becker, 1984.

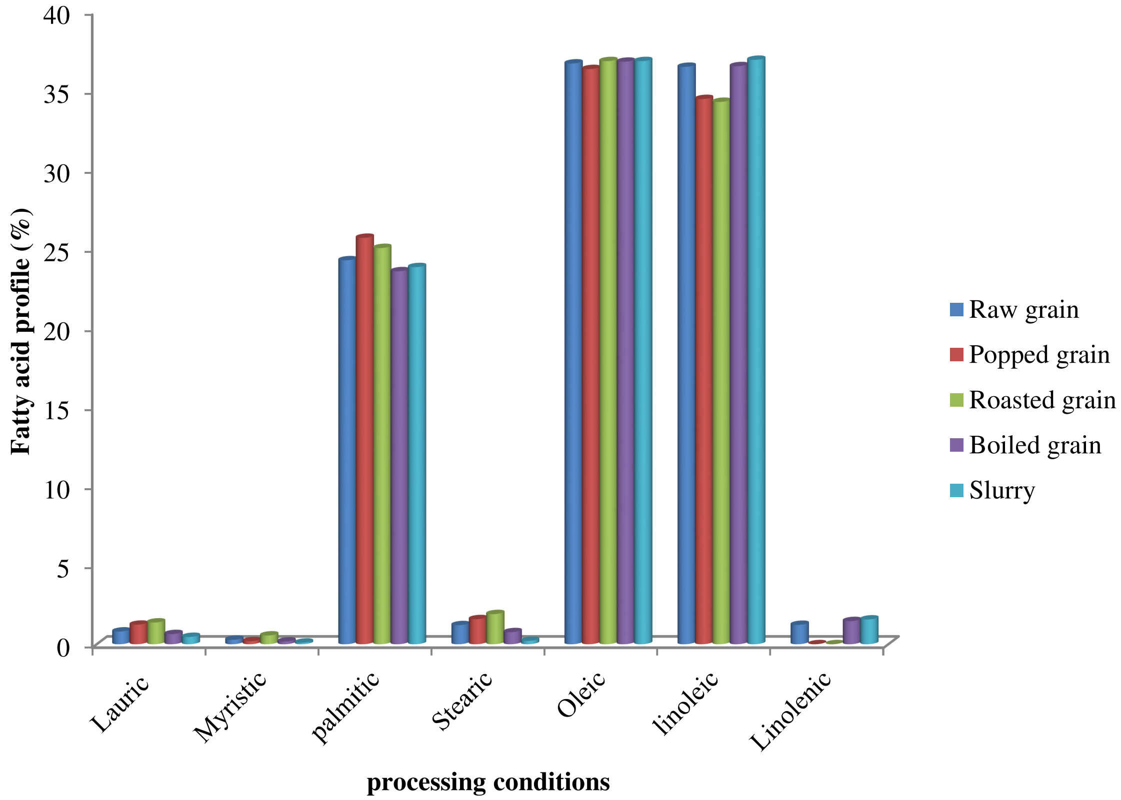

Figure 3: Fatty acid profile (%) of raw and processed grain amaranth

During dry heat (popping and roasting), the level of unsaturation of the oil decreased this is due oxidation of unsaturated fatty acids as they polymerized (Belton and Taylor, 2002). As dry heat is applied, linolenic acids become unavailable as compared to moist heat. It has been reported that water act as a barrier to oxidation and therefore linolenic acid is not oxidized in moist heat. Dry heat techniques should be done with a lot of precautions in order to retain linolenic acid and avoid severe polymerization of oils prior to consumption of the final product.

Generally, Amaranth oil provides an excellent source for omega fatty acids. Berger *et al*., 2003 in a study of the cholesterol-lowering properties of amaranth grain and oil, reported that amaranth oil significantly lowered LDL cholesterol and improved HDL cholesterol, as well as lowering very low density lipoprotein cholesterol (VLDL cholesterol) by 21–50%. Amaranth grains can therefore be recommended as a functional food product for the prevention and treatment of cardiovascular diseases. The inclusion of amaranth oil in the diet contributes to an increase in the concentration of polyunsaturated fatty acids and effective natural antioxidant supplement capable of protecting cellular membranes against oxidative damage (Martirosyan *et al.,*2007).

Conclusion

According to the study, grain amaranth has shown to be a good source of nutrients particularly high protein, fat and minerals. This grain may be used in in mitigating food insecurity in Kenya and at the same time reduce prevalence of malnutrition. Dry and moist heat as processing methods reduced the anti-nutrients content, but moist heat was more effective. It has been shown that water used especially in cooking has an impact on starch gelatinized and protein digested. This helps in utilization of consumed products after preparation. It was demonstrated that moist heating is more effective than dry heating in inducing protein digestibility and starch gelatinization. This implies that availability of nutrients is dependent on processing method used prior to consumption.

Acknowledgements

I wish to express my sincere gratitude and appreciation to Australian Government (AusAID) through, A BecA hub-CSIRO initiative on Food and Nutrition Security for sponsoring and enabling the completion of this research work at Jomo Kenyatta University of Agriculture and Technology.

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